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MATHEMATICAL MODELING OF THE GLUCOSE HOMEOSTATIC SYSTEM IN HUMANS

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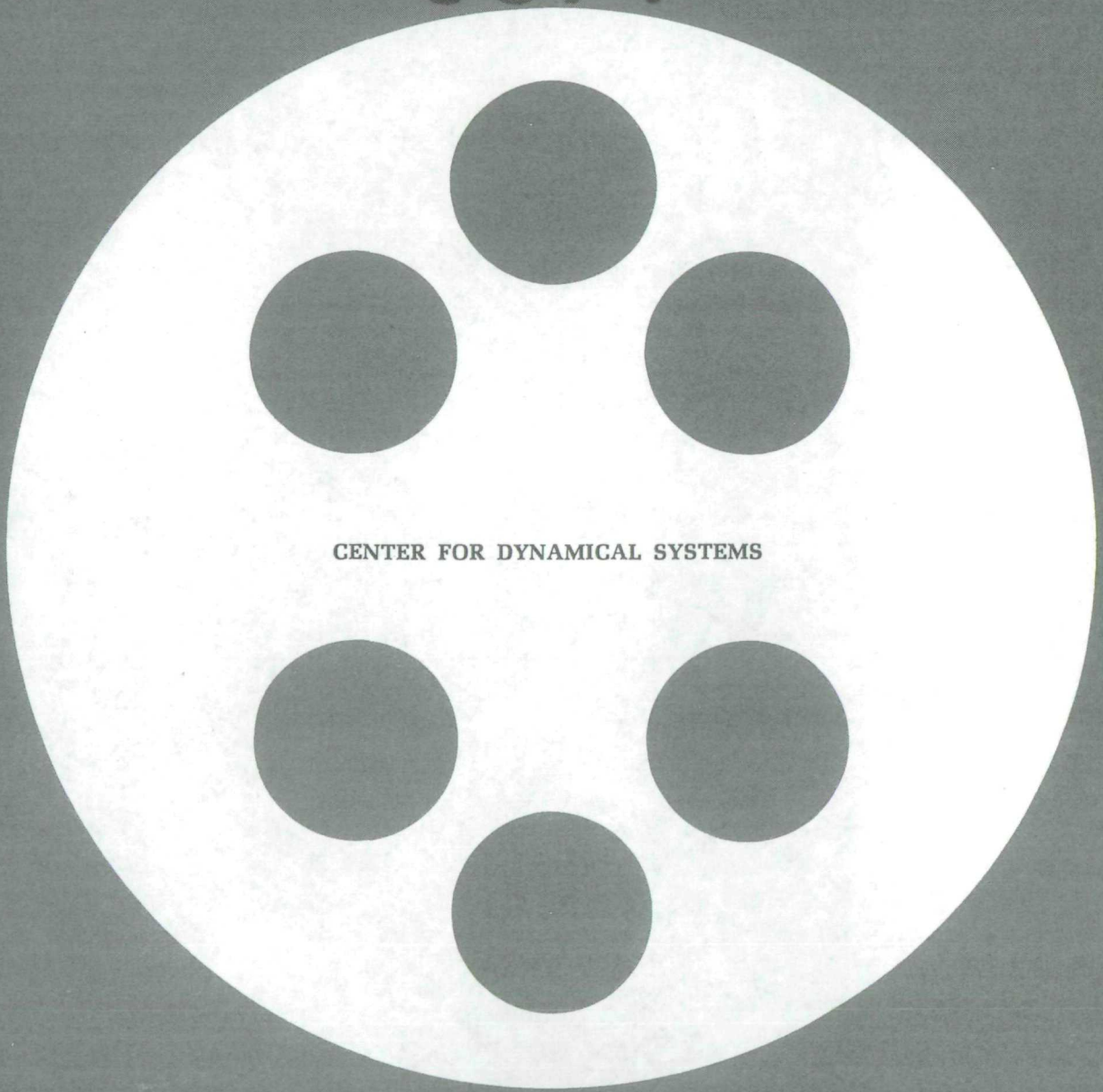

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DIVISION OF APPLIED MATHEMATICS

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MATHEMATICAL MODELING OF THE GLUCOSE HOMEOSTATIC SYSTEM IN HUMANS

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Preface

These notes are based on lectures given by the first author in seminars at the Division of Applied Mathematics, Brown University, at Institut de Recherche D'Informatique et D'Automatique, Rocquencourt, France, and at Laboratoire de Biochimie Médicale, Hôpital Charles Nicolle, Rouen, France and on an honors project completed at Brown University by the second named author. Our interest in modeling of the glucose homeostatic system arose during a joint effort with researchers at Rhode Island Hospital. In fact, Section 3 of these notes contains a preliminary version of a model arising from that joint collaboration. We are deeply indebted to Dr. H. F. Martin and J. Hologgitas and a number of volunteer "subjects" for their substantial contributions to the work discussed in Section 3.

1. Introduction

One of the most interesting physiological control systems in man is the one for glucose homeostasis. Proper functioning of this complex and highly sensitive system is essential for life. Although often thought of as being mainly involved with carbohydrate metabolism, a remarkable organ, the liver, provides a means through which protein metabolism and lipid metabolism play an important role in the glucose homeostatic system. Even a brief perusal of the section on carbohydrate metabolism in biochemistry and/or physiology texts will convince one of the great difficulty in describing this system with a mathematical model which will at the same time be sufficiently simple so as to allow validification with in vivo data and sufficiently complex to provide new insight into the mechanisms involved.

A large number of organs and hormones [25] (see also Figure 1.1) play an important role in glucose homeostasis. A secretion of the β-cells of the pancreas, insulin, has long been recognized as a hypoglycemic factor of prime importance. Insulin secretion is thought to be controlled via signals from the G.I. tract after carbohydrate ingestion, in addition to direct stimulus of the β-cells by glucose. Recent investigations have led to new information on glucagon, a hyperglycemic factor secreted by the α-cells of the pancreas in response to hypoglycemia. Growth hormone (somatotropin) secreted from the adenohypophysis (anterior pituitary gland) not only affects glucose levels in a direct manner, but is believed to interact in a complex

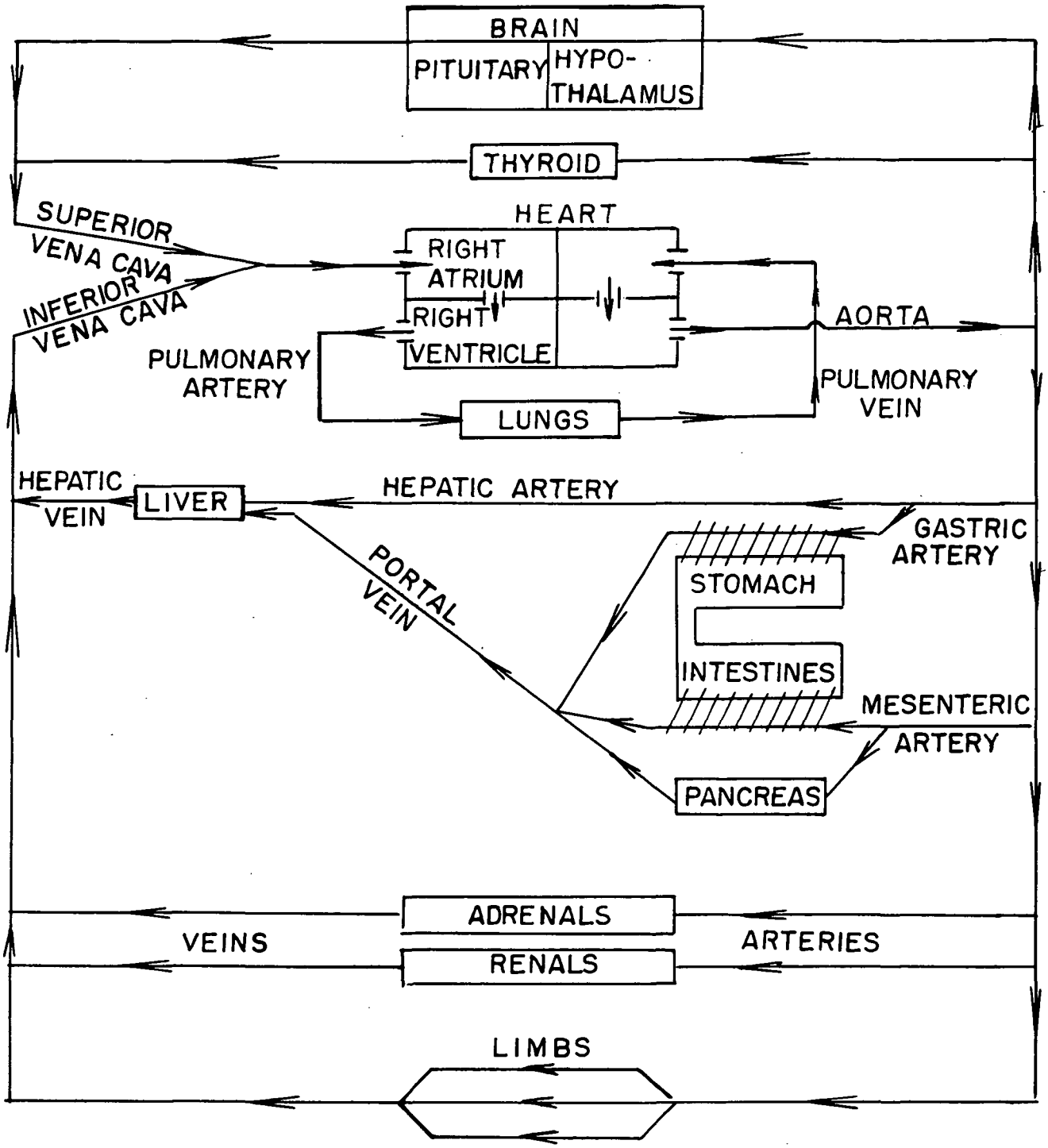
BRAIN
PITUITARY
HYPO-
THALAMUS
THYROID
HEART
SUPERIOR
VENA CAVA
INFERIOR
VENA CAVA
RIGHT
ATRIUM
RIGHT
VENTRICLE
AORTA
PULMONARY
ARTERY
PULMONARY
VEIN
LUNGS
HEPATIC
VEIN
LIVER
HEPATIC ARTERY
GASTRIC
ARTERY
PORTAL
VEIN
STOMACH
INTESTINES
MESENTERIC
ARTERY
PANCREAS
ADRENALS
VEINS
ARTERIES
RENALS
LIMBS

Figure 1.1

manner with other hormones involved in glucose homeostasis, especially insulin. Another secretion of the adenohypophysis, ACTH, stimulates secretion by the adrenal cortex of glucocorticoids (cortisol, etc.) which also play a role in carbohydrate metabolism.

The major site of action of many of these secretory agents is the liver, which is essentially an energy exchange and storage organ. For example, both glucagon and insulin have been implicated in glycogenesis (synthesis of glycogen, the storage form of carbohydrates), glycogenolysis (break-down of glycogen to glucose-1-phosphate) and gluconeogenesis (formation of glucose from non-carbohydrate sources such as glycerol, lactate, amino acids) through their effects on the level of cAMP (cyclic adenosine monophosphate), which inhibits glycogen synthetase and promotes phosphorylation. Another important hormone which affects (elevates, especially in muscle) the levels of cAMP is epinephrine (adrenaline), a secretion of the adrenal medulla which is part of an "emergency" mechanism for quick elevation of plasma glucose in times of extreme hypoglycemia.

Since a major function of the liver involves gluconeogenesis, it is clear that any model of carbohydrate metabolism should include in some way the effect of changing plasma levels of amino acids and free fatty acids (FFA). Thus the mechanisms for break-down and synthesis of lipids (lipolysis and lipogenesis) in adipose tissue are important in any comprehensive modeling attempt. There are other factors, such as thyroxin, which are also believed to play some role in overall control of plasma glucose concentration.

In spite of the complexity noted above, there have been numerous attempts to develop mathematical models for all or parts of the glucose homeostatic system. In the next section we give a brief but incomplete survey on these attempts. We make no effort at describing all previous models, but have tried to make a judicious choice of models so as to illustrate in our discussions a number of differing approaches.

Section 3 contains a preliminary version of a model on which we have been working. Our work is the first, to our knowledge, to offer a model based on simultaneous in vivo measurement of such a large number of the variables involved in glucose homeostasis. We recognize that we still are a long way from a satisfactory model for the physiological control system under discussion here (see the discussions below on the importance of epinephrine, a catecholamine that we have not yet been able to include as a dynamic variable in our model). We are, however, convinced that our approach involving simultaneous in vivo measurement of all variables deserves emphasis.

2. A Survey of Some Previous Models

Wrede [44] was one of the first to propose a model containing most of the parameters which are accepted today as having an important role in the glucose homeostatic system. His goal was a mathematical model, with emphasis on carbohydrate metabolism during glucose tolerance tests (GTT), which would be realistic from a physiological standpoint. The model contained one compartment (plasma) in which variables representing concentrations of glucose, insulin, glucagon, epinephrine, glucocorticoids, thyroxin and growth hormone (x_1 through x_7 respectively) were to be described dynamically by a set of nonlinear ordinary differential equations

$$\dot{x}_i(t) = G_i(x_1(t),\ldots,x_7(t)) + f_i(t)$$

$i = 1,2,\ldots,7$, where f_i are infusion rates. The assumption of only one compartment implies, of course, that secretion response times and mixing times are negligible. Assuming that all variables undergo only small variations about some nominal values, Wrede actually worked with a linearized version of the above system

$$\dot{\delta x}_i(t) = \sum_{j=1}^{7} a_{ij}\delta x_j(t) + a_{i8} + f_i(t)$$

$i = 1,2,\ldots,7$, where a_{i8} are basal production rate constants, a_{ij} is $\frac{\partial G_i}{\partial x_j}$ evaluated along constant equilibrium values of the x_i's, and δx_j

is the perturbation of x_j from its nominal value. The assumption of small variations is invalid since, for example, the typical nominal value for glucose concentration is about 80 to 90 mg % while the concentration varies between approximately 70 to 150 mg % for normal and between 40 and 250 mg % for abnormal responses during a standard GTT.

Wrede used a combination of methods to determine numerical values for the rate constants (coefficients in the system equations) in his linear model including comparison with previous models, determination via physiological in vivo rates in experimental literature, and extrapolation of data from experiments on rat and dog tissue. The assumptions on his vector model

$$\dot{\delta x} = A\delta x + \alpha_8 + f$$

are then such that the 7×7 matrix A has the form

$$A = \begin{pmatrix} & & \tilde{A} & & \\ 0 & \cdots & 0 & a_{66} & 0 \\ 0 & \cdots & 0 & 0 & a_{77} \end{pmatrix}$$

so that x_6, x_7 can be solved for as functions of the infusion rates

f_6, f_7. This essentially allows the model to be reduced to a five dimensional vector system. Using Routh's criterion, the author then establishes stability of the 5-dimensional model with the numerical parameter values he has obtained. In fact, he then obtains the roots of the characteristic equation, yielding asymptotic stability since these all have negative real parts.

Of much greater interest than the stability discussion is Wrede's analysis of the model with respect to accepted physiological "facts" of that time. He makes a number of conjectures based on his study of his model. Some of these, especially those related to basal production rates, appear to be quite good. A number of his conjectures about mechanisms that were unknown at the time have since proved to be quite accurate. A report of simulations with the model is also included. Some of these curves agree qualitatively with data curves usually reported for the GTT, while others are very poor in their approximation to actual responses.

One assumption underlying Wrede's model, that plasma FFA remains unchanged during responses to glucose ingestion, has for some time been known to be false. Shames [37] was, to our knowledge, the first to include plasma FFA levels as an important aspect of a modeling attempt. However, his goal was not development of a model to be used in analyzing responses during GTT's, but instead a theoretical analysis of some of the important variables (glucose, insulin, FFA) in the glucose regulatory system. His aim was a quantitative study of mechanisms involved with an eye

towards a more fruitful laboratory approach to some of the problems involving the system. He analyzed the dynamic characteristics of these mechanisms in terms of responses to constant infusions of glucose. The effects of epinephrine, glucagon, glucocorticoids, thyroxin, and growth hormone are ignored even though the effects of some of these are believed to be mediated through FFA, which is a major component of his study.

One of the most sustained efforts at modeling involving the system under consideration here has been made by Ackerman, Gatewood, Rosevear, and Molnar. In a series of publications [1], [2], [20], [21] they report on attempts at a model in which one or two parameters would yield criteria for distinguishing normal individuals from mild diabetics and prediabetics. Their model is a simplified one, requiring a limited number of data samples during an oral GTT and is not aimed at an isomorphic representation of details of the homeostatic system. We shall give here only a brief summary of their findings. The basic model contains two variables: G, the blood glucose concentration, and H, plasma concentration of a fictitious "composite" hormone (which is to include the action of insulin and other real hormones). Linearization of a general nonlinear model about nominal values G_o, H_o leads to non-homogeneous linear equations in g and h, the variations from the nominal values. That is, one has

$$\dot{g} = -m_1 g - m_2 h + J$$
$$\dot{h} = -m_3 h + m_4 g$$

where $g = G - G_o$, $h = H - H_o$ (see Figure 2.1), and J is the input function.

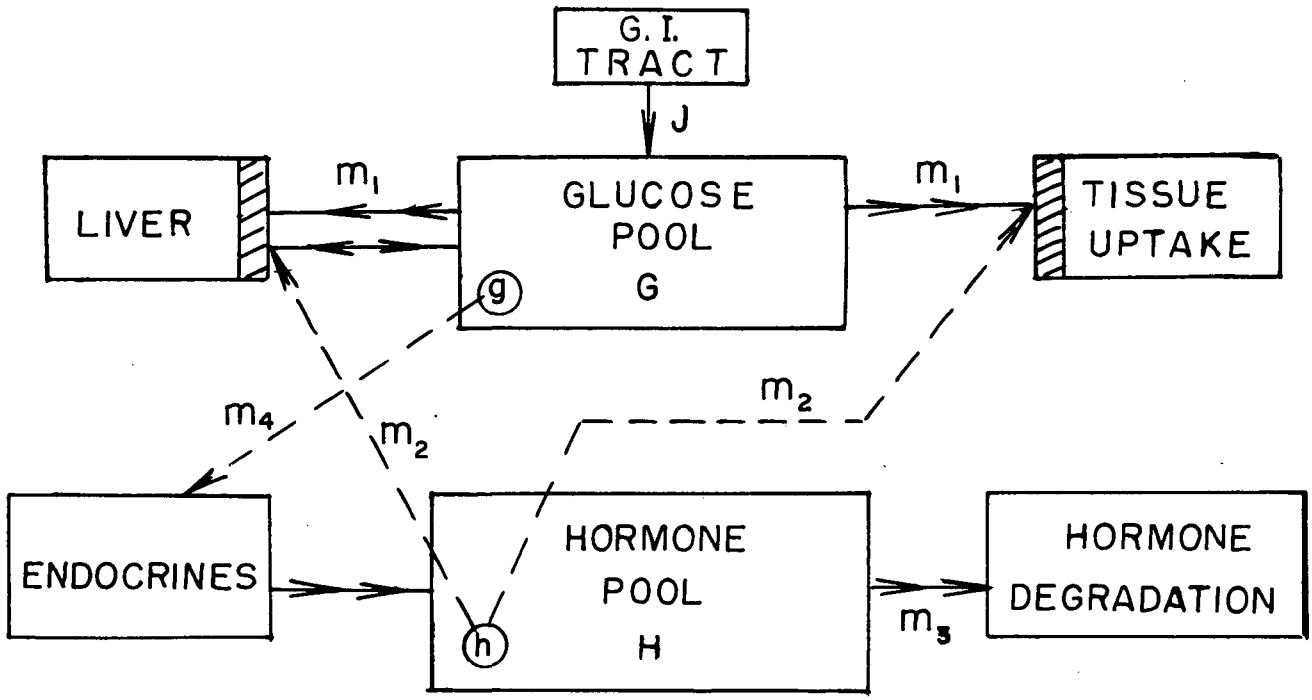

Figure 2.1

Combination of these two first order equations in the usual manner yields an equation for g of the form

$$\ddot{g} + 2\alpha\dot{g} + \omega_o^2 g = S$$

where $\alpha = \frac{1}{2}(m_1+m_3)$, $\omega_o^2 = m_1m_3 + m_2m_4$, and $S = m_3J + \dot{J}$. Solutions to

this system, which can be given in terms of damped oscillations $e^{-\alpha t}\sin(\omega t+\phi)$, $\omega = \sqrt{|\alpha^2-\omega_o^2|}$, and the parameters of S (i.e., J), were found to agree reasonably well with the damped oscillatory responses observed in many patients.

The authors studied the system as a function of the parameters m_1, m_3, ω and the parameters of J, and compared simulations with data collected at the Mayo Clinic. In fitting this model to data they found that although unique values of the parameters could be obtained, variations in one parameter value could easily be compensated for by variations in the values of another parameter. Furthermore, the values for α and ω proved to be highly sensitive to small errors in blood glucose concentrations. However, they did determine that one parameter, the natural frequency ω_o, is relatively insensitive to experimental error and variations in the other model parameters. Thus they are able to take $T_o = 2\pi/\omega_o$ as the basic description of responses to the OGTT. Using data from a variety of sources, Ackerman, et.al. concluded that responses with T_o substantially less than four hours are characteristic of normal responses, T_o substantially larger than 4 is indicative of mild diabetes while responses with $3.5 < T_o < 4.5$ appear to be in a grey area with respect to diagnosis.

The results of these studies are supported by the findings of Ceresa, et.al. [10] who have taken the same type of two variable model approach. The main difference between their model and that described above is their assumption that h actually represents concentrations of insulin, not a composite hormone. Ackerman, et.al. have pointed out that simulation studies of the variable h yield a fit to insulin data

only during the first two to three hours of the five hour OGTT.

Ackerman, Gatewood, Rosevear and Molnar admit that there are a number of detrimental aspects associated with their two-variable modeling approach. First, one will never obtain in this way a model isomorphic in any realistic sense to the physiological control system. A second difficulty involved inabilities to simulate responses with biphasic initial peaks. Finally, there were some problems in fitting the glucose concentration simulations to data in the time period between three hours and five hours after ingestion. As Ackerman, et.al. suggest and as our own work reported below establishes, there are other (hyperglycemic) factors such as glucagon, growth hormone, and perhaps epinephrine, which play an important role in the control system during this time period.

Antomonov, et.al. [3] have recently described a model (see Figure 2.2) based on components consisting of various organs and sites in the circulatory system, the mechanisms involved in each component being represented by simple linear ordinary differential equations. In this three variable model (glucose, insulin, epinephrine) the authors take a black-box input-output approach and offer only limited physiological justification in writing the equations which are detailed below.

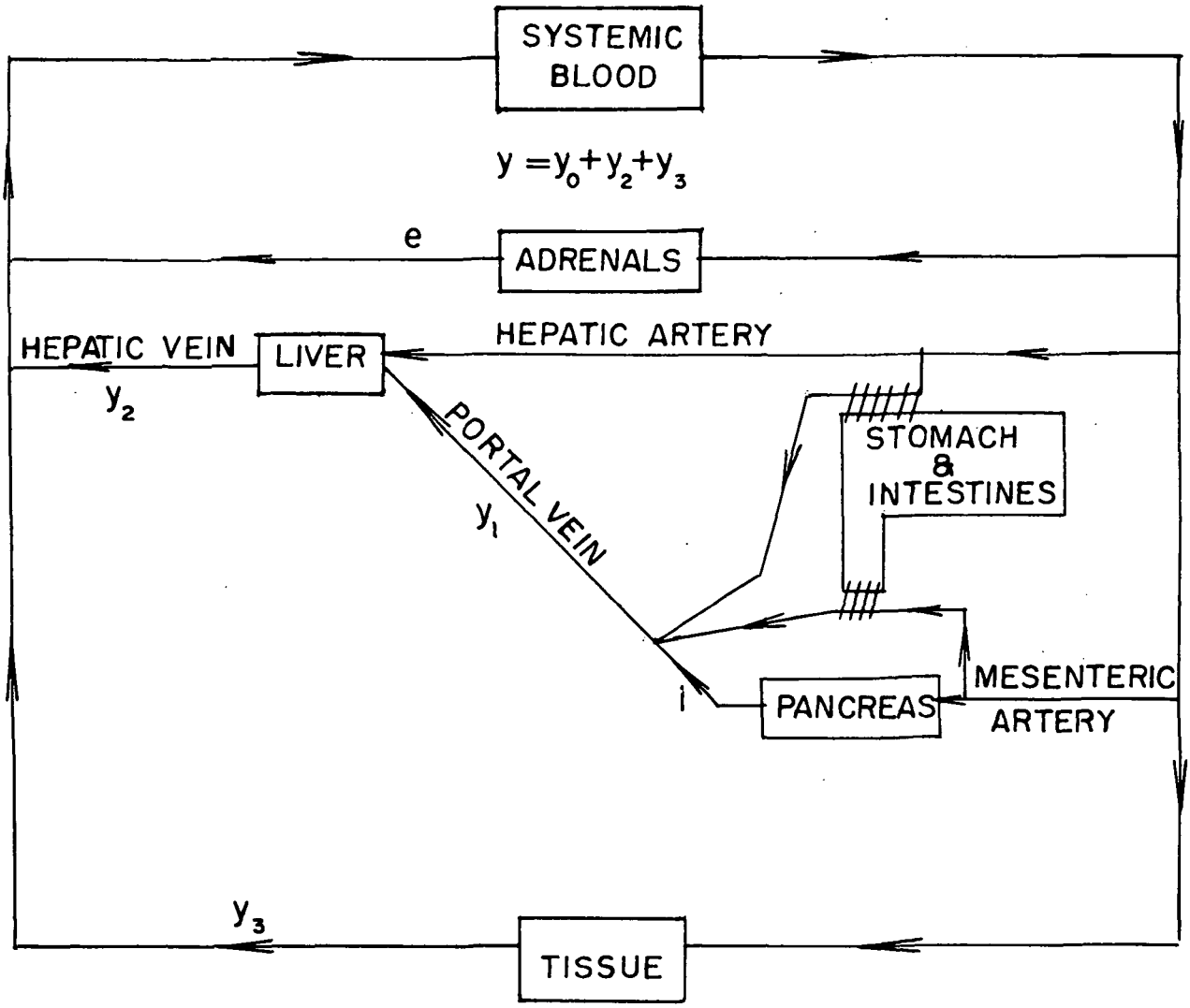

Figure 2.2

G.I. tract to portal vein:

$$\dot{y}_1 = -a_1 y_1 + k_1 r_1 \chi_{[0,\tau_a]},$$

where r_1 is the absorption rate from the stomach and intestines into the portal vein, χ_A is the characteristic function of A, and y_1 is the deviation in the portal vein from "normal" or equilibrium glucose concentration y_0.

Liver:

$$\dot{y}_2 = a_2 y_1 - a_3 y_2 - a_4(y-y_o) - b_1 i + c_1 e,$$

where i and e are the circulating levels of insulin and epinephrine, y is the systemic concentration of glucose, and y_2 is the deviation in the hepatic vein from the normal glucose concentration.

Pancreas:

$$\dot{i} = -b_2 i + a_5(y-y_o),$$

where this equation is assumed to hold only when $y - y_o > 0$.

Adrenals:

$$\dot{e} = -c_2 e + a_6(y_o-y),$$

where again this holds only in the case $y - y_o < 0$. Both this equation and the previous one obviously assume that there are sensory mechanisms for the systemic concentration y involved in the controls of the pancreas and adrenal glands.

Peripherel tissue:

$$\dot{y}_3 = -b_3 i - b_4 i_1 - a_7 y_3,$$

where i_1 is systemic insulin received by injection into muscle tissue and y_3 is the glucose concentration at the tissue output.

Injected insulin:

$$\dot{i}_1 = -b_5 i_1 + k_2 i_1^{(1)} \chi_{[0,\tau_i]},$$

where $i_1^{(1)}$ is the rate of resorption due to blood flow from the area of tissue where the injection was made.

By solving the above system of equations and fitting the solutions to experimentally obtained curves, the authors determined values for the parameters a_j, b_j, c_j of the model. They then tested the model by simulation of pathology via parameter perturbation. That is, they asked if one could, by making changes in parameters appropriate to pathologies, obtain solutions of the model which simulate responses characteristic of individuals suffering those pathologies. Some success along these lines is reported in testing the pancreatic insulin mechanism in the model with regard to diabetes and insulinemia. Also included are the results on attempts to apply optimal control theory to choose doses (minimum time criteria) of glucose and insulin for treatment during pathology. Finally, a study of the glucose homeostatic system during embryogenesis showed that evolution of the control mechanism could be paralleled by changing ("evolving") the parameters in the model, the dynamical curves thus produced by the mathematical model agreeing well with experimental curves. This, the authors argue, offers substantial support for the correctness of their model.

An excellent review of some modeling attempts through 1967 which includes comments on the models of Wrede, Shames, and Ackerman, et.al. described above can be found in [12]. The authors there comment on the obvious need for nonlinearities in the models (this agrees with our findings reported in Section 3 below). Such a nonlinear mathematical model, based on the Ph.D. theses of Charette [11] and Srinivasan [39], is detailed in [40]. The authors' goal is to reproduce the gross effects

of metabolic interaction of FFA and glucose in response to IV infusions of glucose, insulin, etc. A short term (two hours) model comprised of many interconnected subsystems which have been modeled on the basis of known stimulus response characteristics is formulated. The emphasis is on a nonlinear systems approach, with validation of the model via clinical data available to the authors at the time. An interesting feature of this model is the extensive use of the hyperbolic tangent function in connection with switching mechanisms. As the authors point out [40, p. 159], "although the model does not adequately reproduce the finer details of the actual responses in some instances, the limited success supports the systems approach to the problem". They contend that the systems analyst has a role in the development of knowledge of the physiology of relevant subsystems, contributing to this effort "by systematizing the known physiologic facts about the metabolic processes in a consistent mathematical framework, by proposing to the physiologist specific experiments suggested by model building, simulation and analysis, and by determining analytically the consequences of conflicting theories on the operation of specific portions of the overall plant".

We mention one other recent survey paper of interest here. In [4] Atkins presents a survey of 24 (some quite different) theoretical models for regulation of plasma glucose concentration via insulin. The author's purpose is to "see whether it is valid to propose a model and fit it to plasma glucose levels only". His conclusion, based on data fitting and simulation, is that it is impossible to distinguish between

and assess the validity of a large number of models when only glucose data is used to fit the models. This supports fully our own view of the extreme importance in collecting (simultaneously) data from each subject for all (or as many as feasible) of the variables involved during an OGTT.

3. A New Model

In this section we report on a model for glucose homeostasis being developed by H. T. Banks and Christin A. Carter of the Division of Applied Mathematics, Brown University and H. F. Martin and J. Hologgitas of Rhode Island Hospital. Our goals include a comprehensive mathematical model to be used as an aid in teaching and diagnostic efforts in relation to patients suffering from hormonal disorders. As with most attempts at modeling, we hope that a systematic approach based on current physiological information and data from subjects tested at Rhode Island Hospital will lead to a better understanding of the complex physiological control system involved in homeostasis.

After an overnight fast, subjects were given an oral dose of 100 gms of glucose and required to remain at rest for a five hour period. Blood samples were taken every twenty minutes in quantities large enough to permit simultaneous measurement of plasma levels of glucose (x_1 in the model below), insulin (x_2), glucagon (x_3), growth hormone (x_7), and free fatty acids (x_8). Our initial efforts also included checks on plasma levels of cortisol (x_5), thyroxin (x_6), and amino acids (x_9), but we found that these remain fairly constant during the five hour test. Thus these quantities are assumed constant in the model described below.

Our attempts with a linear model were unsuccessful and a preliminary form of the nonlinear model discussed here contains functions of the following type:

$$S(x;a,b) \equiv \begin{cases} 0 & \\ (x-a)/(b-a) & a \leq x \leq b \\ 1 & \end{cases}$$

$$H(x) \equiv \begin{cases} 0 & x < 0 \\ 1 & x \geq 0 \end{cases}$$

$$Q(x;a,b) \equiv \begin{cases} 1 & x < a \\ (b-x)/(b-a) & a \leq x \leq b \\ 0 & x > b \end{cases}$$

$$L(x;\alpha,A,m) \equiv \begin{cases} (A/\alpha)x & 0 \leq x \leq \alpha \\ A + m(x-\alpha) & x > \alpha \end{cases} .$$

It is obvious that the piecewise linear functions S,Q are meant to be only rough approximations to the sigmoid curves often found in saturation-limited phenomena and data. While the actual nonlinearities are no doubt smoother and more complex, these approximations are extremely convenient to use when one is running simulations on the computer while varying the shapes of the curves through variation of the parameters a and b.

The model, for "normal" responses to the oral glucose tolerance test (OGTT), is basically a one compartment model (systemic plasma) although as it will be seen below, we have taken some care in determining uptake and output terms for the variables in the model at various organ and tissue sites in the body. We give here each equation of the model separately along with a brief discussion of underlying assumptions and justifications.

Glucose

$$\dot{x}_1(t) = -a_{127}L(x_2(t);\alpha,A,m)S(x_1(t);\alpha_{11},\beta_{11})Q(x_7(t);\alpha_{17},\beta_{17})$$
$$-a_{12}x_2(t) + a_{13}S(x_3(t);\alpha_{13},\beta_{13}) + a_{18}S(x_8(t);\alpha_{18},\beta_{18}) + f_1(t) + r_1$$

The first term on the right side of the equation represents uptake of glucose by extrahepatic tissue (mainly muscle and adipose tissue) which is thought to be rate-limited at the cell membrane. Capillary and interstitial levels of glucose are assumed the same since it is believed that diffusion rates of glucose across capillary walls into interstitial fluid are much higher than utilization rates [15]. The absence of intracellular free glucose [25, p. 238] justifies the assumption that entry into the cell and not utilization (phosphorylation) is the most important factor with respect to rate limitation.

It is well-known [25, p. 238] that insulin facilitates glucose uptake in adipose tissue and muscle. In fact, the insulin concentration of arterial blood has been shown to be the primary factor involved in stimulation of glucose uptake in intact muscle [13], with the only site of direct action being the cell membrane [25, p. 240]. The function L used above for the action of insulin can be considered an approximation to a Langmuir type curve. The Langmuir isotherm curve [28] is derived in connection with the adsorption of gases on solids. Extrapolation of this theory to the attachment of insulin molecules to cell membranes (concentration of insulin is then equated to gas pressure) involves the following assumptions: transport of glucose is dependent upon the number of sites occupied by insulin; there are a limited number of sites; and insulin facilitates transport only when it is attached to the cell membrane. That Christensen and Ørskov [13] found a maximum insulin

effect (at about 200 μU/ml) on muscular glucose uptake lends support to the above extrapolation. We note that our approximation to the Langmuir curve is made up of only two linear functions even though in fact a third function (roughly a constant) should be included for values of x_2 larger than about 200 μU/ml. We have not needed to include this in this preliminary model since our data from normal subjects to date shows no times at which this level is exceeded.

Insulin levels are not the only limiting factors with regard to the entry of glucose into extrahepatic cells. Experiments (in vitro and in vivo) indicate that glucose itself has a stimulatory effect on muscular glucose uptake [13]. Even if insulin levels do not rise with an increase in blood glucose, glucose entry will increase due to a rise in substrate level [25, p. 238]. The glucose term incorporated in our extrahepatic uptake term is saturation limited at both ends. This simply is indicative of the fact that if glucose blood levels fall too low, there will be a negligible amount of uptake in muscle and adipose tissue regardless of the levels of insulin while on the other hand, the amount of glucose available is no longer considered a limiting factor when glucose levels are sufficiently high. A simple diffusion term of the type $-a_{11}x_1$ based on glucose levels (and not involving insulin) cannot be supported physiologically (see the above comments on diffusion of glucose across the capillary walls). Furthermore, our attempts to incorporate a term of this type in the model were not successful when model simulations were compared with our data.

A third factor which affects significantly the rate of glucose uptake in extrahepatic tissues is growth hormone, which tends to

"block" insulin [5], [13], [18], [32], [33], [46]. It is believed that growth hormone decreases the effectiveness of increased insulin levels in promoting glucose uptake by decreasing the sensitivity of muscle and adipose membrane to insulin [33]. It may do this either by interfering with the transport mechanism [25, p. 242] or perhaps by decreasing the tissue binding of insulin [19, p. 261]. An additional or alternative mode of action is suggested by the report [19, p. 261] that growth hormone, in the presence of glucocorticoids, has been found to elevate plasma levels of protein factors that antagonize the action of insulin in vitro.

In contrast with the situation in muscle and adipose tissue, transport across the cell membrane is not the rate limiting factor in glucose uptake in tissues of the brain, kidney, gastrointestinal tract, or liver [25, p. 176], [34, p. 700]. Uptake and output in the liver is quite complicated and will be discussed in detail below. Uptake in the tissues of the brain, kidney, and G.I. tract appears to be relatively independent of hormonal variations. In this model we have included this constant uptake in the term r_1. A more realistic approach would use a function $S(x_1; \alpha,\beta)$ with α very small and β somewhere between 40 and 60 mg %. This was unnecessary in our work since the values of x_1 seldom fall below 60 for subjects with a normal response to an OGTT.

The liver, the most complex of the organs involved in glucose homeostasis, is capable of (i) uptake of glucose and its conversion to glycogen (glycogenesis), (ii) conversion of glycogen to glucose to be released into the blood (glycogenolysis), and (iii) conversion of

noncarbohydrate substances such as glycerol, lactate, and amino acids to glucose (gluconeogenesis). It is useful (and accurate) to picture the turnover of glucose by the liver (i.e. glucose to glycogen and glycogen, etc., to glucose) as constituting a state of "dynamic equilibrium" in "resting" (i.e. x_1 unperturbed) situations. That is, uptake and output by the liver is occurring at a high rate, but is balanced so that the net effect on blood glucose levels is zero. Lowered or raised levels of glucose in the blood affect this balance by changing the rates of uptake and/or output.

One controller of this rate is insulin, which when increased has the net effect of decreasing output of glucose by the liver [8], [19, p. 257], [25], [35], [36]. It is believed that insulin achieves this result by lowering the intracellular levels of cAMP, which is involved in the metabolism of glucose in at least two important ways. First of all, cAMP activates phosphorylase kinase kinase which then activates phosphorylase kinase. This in turn causes the phosphorylation of inactive glycogen phosphorylase to yield the phosphorylated or active form. The active form promotes the breakdown of glycogen to glucose-1-phosphate. Thus, lowering cAMP levels inhibits glycogenolysis and decreases the direct output of glucose by the liver. A second way in which cAMP acts in the liver is through the deactivation of glycogen synthetase. This is effected through the stimulation of glycogen synthetase kinase which catalyzes the conversion of an active form of glycogen synthetase to a less active form. Hence decreased intracellular cAMP concentrations lead to reduced inactivation of glycogen synthetase and an increased rate of glycogenesis.

It has been shown [6], [24], [35, p. 326] that insulin also affects

significantly the levels of cAMP in adipose tissue, and to a much lesser degree, the levels in muscle tissue. As will be discussed later, insulin thus plays an important role in regulation of lipolysis in adipose cells. From a modeling point of view, the insulin-mediated changes in cAMP levels in adipose and muscle cells probably play a secondary role to the rate-limiting transport at the cell membrane involved in glucose uptake by these tissues (although changing cAMP levels may play an important role in the transport phenomenon [24, p. 762]). Therefore, the second term on the right side of the above equation can be considered as representing the hepatic role for insulin levels with respect to glucose uptake. Also included in this term is the known effect of insulin in reducing gluconeogenesis [7], [25, p. 180], [25, p. 241] which may also be attributed to a direct action of cAMP or may simply be secondary to the decreased glycogenolysis since glycogen depletion accelerates hepatic deamination and transamination reactions [19, p. 208].

In direct opposition to the effects of insulin on glucose metabolism in the liver are those of glucagon. Glucagon has been shown to increase glucose output from the liver by increasing glycogenolysis and gluconeogenesis [7], [16], [41] and to decrease glucose uptake by inhibiting glycogenesis [19, p. 259], [38]. Evidence indicates that glucagon activates the enzyme adenyl cyclase [24], [25] which is found in the cell membrane. This enzyme catalyzes the reaction of ATP to cAMP [25] which acts to increase glycogenolysis, decrease glycogenesis, and perhaps effect the observed increase in gluconeogenesis (see the above comments on cAMP and gluconeogenesis). With the possible exception of myocardium, glucagon does not stimulate muscle glycogenolysis

[25, p. 238]. In fact, its action appears to be limited to the liver.

Growth hormone may also contribute to increased hepatic-glucose output [19, p. 261] although current supporting evidence is far from conclusive. Attempts at including a term for this action in the model were not successful when used with our data. One possibility is that the effects of growth on the liver are delayed as in the case of its stimulation of lipolysis (see the discussion on the FFA equation below) so that its effect is not seen in a five hour OGTT.

Another contribution to increased glucose levels involves increased levels of FFA which is related to stimulation of gluconeogenesis. Two possibilities have been suggested in regard to this stimulation. The first involves the concomitant release of glycerol with FFA during lipolysis at fat cells. This glycerol acts as a substrate for gluconeogenesis, being converted to phosphoglyceraldehyde which enters the Embden-Meyeroff pathway for glycolysis [19, p. 202], [25, p. 180]. Thus increased levels of FFA are indicative of increased levels of glycerol which are available to play the role of a substrate. The FFA's themselves may play a direct role in gluconeogenesis by inhibiting pyruvate oxidation, stimulating pryuvate carboxylation, and promoting malate formation [42].

The role of FFA-stimulated gluconeogenesis is not considered of major importance during the GTT [19, p. 203]. This agrees with our findings that the FFA term should enter the model only when glucose levels are relatively low and FFA levels are comparatively high (above approximately .4 mE/ℓ). In the model we have assumed this effect to be saturation limited at both ends.

Of the other hormonal controllers involved in glucose regulation,

epinephrine is the most important. It appears to act in much the same way as glucagon, although it is effective in muscle and adipose tissues rather than at the liver [25, p. 177]. Like glucagon, it stimulates adenyl cyclase which increases the concentration of cAMP, resulting in the observed increases in glycogenolysis and decreases in glycogenesis [25], [31]. Epinephrine may also be effective in two other ways: by directly inhibiting glucose uptake by muscle [31] (in which case it should be included in the model in the nonhepatic uptake term), and by stimulating the Cori cycle [25, p. 238], a process involving conversion of lactate to glucose in the liver.

Epinephrine is normally regarded as an emergency rather than as a continuous controller in homeostasis [25, p. 241]. Consequently, it may not be of great importance during the OGTT. However, evidence indicates that levels of epinephrine may rise dramatically during the recovery phase of the OGTT response when glucose levels have been lowered below fasting levels. Unfortunately, no reliable methods have been developed to measure epinephrine and its effects were by necessity treated as nonvarying in our model.

Thyroxin is another hormone which plays a role in glucose metabolism, effecting increased gluconeogenesis in the liver and increased glucose oxidation in tissue [34]. Since thyroxin levels do not change substantially during the OGTT, this contribution can be included in the constant term r_1 in the model.

Experimental evidence indicates that the presence of another group of hormones, the glucocorticoids, is necessary for the action of glucagon, epinephrine, and growth hormone in glycogenolysis and gluconeogenesis [7, p. 673], [25, p. 180], [25, p. 241]. Whether the adrenal cortex and

its secretions play other than a "permissive" or "conditioner" role in facilitating glucose metabolism remains uncertain. These effects have been treated as a constant in the model since plasma levels of the best known glucocorticoid (cortisol) do not vary significantly during the OGTT.

Two final aspects of the glucose equation remain to be discussed: the input function $f_1(t)$ and a renal clearance term. Relatively little is known about absorption of materials across the gut so that modeling of absorption is a significant problem in itself [21]. Based on our limited experience and that of others, we have assumed an absorption across the gut of approximately 70% of the total amount of glucose ingested. The input to the plasma has, to date, been approximated by a piecewise linear continuous function consisting of three sequential parts: (i) a steep rise to a maximal absorption rate (ii) constant maximal absorption and finally (iii) a sharp drop of absorption to the zero level. T he period of time for the sections (i), (ii), (iii) must naturally vary with the individual subject. A rough estimate on the ranges of these periods would be about 20-45 minutes for phase (i), followed by approximately 1-2 hours of maximum absorption rate, then a fall to zero by 2 to 3.5 hours after ingestion.

Although most textbooks give figures of approximately 160-180 mg % as the renal threshold level for glucose excretion, work and experience at Rhode Island Hospital indicate that renal excretion will not be a significant factor unless glucose levels reach approximately 300 mg %. This level is never attained by subjects with a normal response to the OGTT and hence we have not included such a term in our model. Suggested procedures for the OGTT include collecting urine at the end of the 5 hour OGTT, after having had the patient empty his or her bladder

immediately prior to the ingestion of the glucose. If an appreciable amount of sugar is then found in the urine, an $S(x_1; \alpha,\beta)$ term with $\alpha \approx 300$ should be included in the model (the other parameters in this term are chosen so as to be consistent with the total amount of sugar excretion indicated by the sample).

Insulin

$$\dot{x}_2(t) = a_{21}(x_1(t) - \gamma_{21})H(x_1(t) - \gamma_{21}) + \bar{a}_{21}\dot{x}_1(t)H(\dot{x}_1(t) - \bar{\gamma}_{21}) - a_{22}x_2(t) + r_2$$

Insulin levels in the systemic blood are raised in response to increased levels of glucose. The feedback effect of blood glucose levels acting directly on the pancreas [19, p. 257], [34, p. 702] is considered to be the major control of insulin secretion, and is felt by some to affect insulin release rather than insulin synthesis. However, there is growing support for the "two-pool" model [25, p. 176] for insulin secretion consisting of (i) rapid release of insulin from a small "pool" or compartment of the β-cell and (ii) continuous release coupled with synthesis. The first term on the right side of the above equation arises from the assumptions that when glucose levels are normal or low, secretion of insulin is at a constant low rate (included as a part of r_2). When the level of glucose is elevated above fasting level, insulin secretion is increased [19, p. 257].

We have also included a term involving the rate of increase of glucose concentration as a stimulus for insulin secretion. Although no direct physiological support for this has yet been found, other authors who have dealt with glucose homeostasis have suspected this

phenomenon [40]. Work at Rhode Island Hospital supports this type of term in principle, although no specific in vitro or in vivo experiments have been performed yet. The "two-pool" theory explained above may lend some support to this assumption, the rapid release in (i) being associated with an $\dot{x}_1$-type term, while (ii) is modeled by the term involving levels of x_1. The mechanism for the rapid-release pool is not yet completely understood, but may be related to the "glucagon-like" substance believed to be released by the intestinal tract as an "early-warning" or pre-hyperglycemia signal to the β-cells of the pancreas. While this may not be exactly an $\dot{x}_1$-type signal, sensors of some type which involve flux of glucose across the intestinal mucosa appear to be indicated.

In our previous discussion of disappearance of glucose from the plasma in nonhepatic uptake, we found that the rate-limiting factor was transport across the cell membrane (hence the complicated term involving insulin, glucose, and growth hormone). In contrast the rate-limiting factor in the depletion of systemic insulin at extrahepatic sites appears to be its diffusion from capillaries into interstitial fluid. In fact, experimental evidence involving adipose cells indicated a necessity of plasma levels of insulin ten times those needed in interstitial fluid to produce the actions attributed to insulin [43]. In addition, the liver breaks down and inactivates insulin via the "insulinase" system. The rate of this degradation also appears to depend upon circulating insulin levels. We have, therefore, combined hepatic and nonhepatic "degradation" of insulin in the $-a_{22}x_2$ term.

With the possible exception of epinephrine (which is believed to

act directly on the pancreas to inhibit insulin secretion [19, p. 258]), the effects of other hormones upon insulin secretion and/or disappearance remain questionable. Growth hormone interferes with the action of insulin at the cell wall (as noted above, this is not rate-limiting in the disappearance) but probably has no direct effect on insulin degradation. Secretion may be enhanced, but the mode of action is unclear since it has not been possible to show a direct in vitro effect of growth on isolated pancreatic tissue [25, p. 242]. Glucagon has been found to stimulate insulin secretion by direct action on the β-cells, but its potency has been questioned [14].

Glucagon

$$\dot{x}_3(t) = a_{31}Q(x_1(t);\ \alpha_{31},\beta_{31}) - a_{33}x_3(t) + r_3$$

Not much is known concerning the secretion and degradation of glucagon, the hormone secreted by the α-cells of the pancreas. Only recently has a reliable method for measuring levels of glucagon in the blood been developed and there is much current activity on the mechanics related to this hormone. Even though the theories of Unger and his coworkers [25, p. 241], [30], [41] may not yet be widely accepted, the evidence collected thus far clearly suggests the hypotheses that hypoglycemia and fasting promote the secretion of glucagon while increased blood glucose levels suppress its secretion. The method of control is thought to resemble that of insulin; e.g., a sensory mechanism in the α-cells is triggered directly by stimulation from circulating glucose and not by neurogenic (CNS) signals [34, p. 704]. It is thought that the α-cell is "set" to secrete glucagon at a high

rate (just as β-cells are "set" to secrete insulin at low rates) during the basal fasting state. Increasing glucose levels suppresses the secretion of glucagon to some minimal rate [41, p. 444]. In our model we have represented this with a term of the form $a_{31}Q(x_1) + c$, the constant c being included in the r_3 term and being the minimum level of secretion.

As we have pointed out above, glucagon affects glucose metabolism at the liver. The observations that circulating glucagon has such a short half-life (a matter of minutes) [19, p. 259], that a large proportion of endogenously secreted glucagon is bound in the liver, and that, in beef liver at least, an enzyme is found which degrades glucagon, indicate that the liver itself helps to regulate the level of glucagon in the blood. It appears that there is significant degradation of glucagon based on circulating levels. This is modeled by the second term on the right in the above equation.

In addition to the component already mentioned, the term r_3 may contain other (known, or as yet unknown) constant factors affecting the secretion of glucagon. For example, it is already suspected [25, p. 241], [30], [41] that increased levels of amino acids provoke added glucagon secretion. Since amino acid plasma levels were relatively unchanged during the OGTT, such an amino acid term would be included as a part of the r_3 term.

Growth Hormone

$$\dot{x}_7(t) = a_{71}H(\gamma_{71} - x_1(t)) - \bar{a}_{71}\dot{x}_1(t-4/3)H(-\dot{x}(t-4/3))H(\bar{\gamma}_{71} - x_1(t)) - a_{77}(x_7(t) - e_7)$$

The principal controllers of human growth hormone (HGH) levels in

the systemic blood appear to be the levels of glucose and HGH itself. During hypoglycemia one usually has an increase in the plasma levels of HGH [25, p. 242], [45]. It is now thought that lowered levels of glucose stimulate receptors in the hypothalamus which cause secretion of a Growth Hormone Releasing Factor (GHRF) to the adenohypophysis, which then in turn releases HGH into the blood [19, p. 301], [46, p. 76]. In support of such a hypothalmic control mechanism, it has been shown that infusions of glucose limited to the hypothalamic region prevent the expected increase in systemic HGH following hypoglycemia [19: 3rd ed., p. 333]. Furthermore, both crude and purified extracts of hypothalamic tissue stimulate the release of HGH from the adenohypophysis [34, p. 686]. Since it is not clear that lowered levels of glucose do anything other than signal release of GHRF, we have modeled this strictly as a triggering term.

A simple secretion based solely on glucose levels alone does not, however, come close to describing the data collected from subjects at Rhode Island Hospital. It is likely that secretion of HGH is also stimulated by falling glucose levels [25, p. 242], [26], [45]; we have represented this conjecture by the rate term involving $\dot{x}_1$. Since it is suspected that the greater the rate of fall, the greater the rise in HGH secretion [26], we have included $\dot{x}_1$ itself as a proportionality factor. That this mechanism effects a delayed response (as seen by observing systemic HGH levels - see also [22, p. 431]) is supported by our findings with subjects (most had a relatively flat HGH curve until about 3.5 hours at which time it abruptly rose, approximately .5 to 1.5 hours after a dramatic fall in glucose). This (slightly delayed) increased secretion is characteristic of the recovery phase [25, p. 242]

of a response to the OGTT. To explain this phenomenon, we have hypothesized that the rate of glucose fall is a potent stimulus for secretion of HGH only when the level of glucose is such that the fall may lead shortly to frank hypoglycemia. Subjects exhibited significant drops in glucose levels without an HGH stimulus only in periods where the level of glucose remained above equilibrium (fasting) levels.

Support for a "negative feedback" mechanism in controlling HGH levels in the blood is readily available [19, p. 302], [29], [46]. It is thought that this "feedback" is effected by the pituitary gland itself through monitoring of HGH plasma concentrations (the hypothalamus is not implicated here). We have represented this by the last term involving an "equilibrium" value e_7 which is usually approximately 1.0 to 1.5 ng/mℓ.

It is clear that the control system for HGH is more complex then the ones we have discussed for the hormones secreted by the pancreas. Thus should not be surprising in light of the fact that the control system is known to involve at least the CNS, the hypothalamus, and the pituitary gland itself.

Free Fatty Acid

$$\dot{x}_8(t) = -a_{82}S(x_2(t);\ \alpha_{82},\beta_{82}) + a_{83}S(x_3(t);\ \alpha_{83},\beta_{83}) - a_{87}S(x_7(t);\ \alpha_{87},\beta_{87}) - a_{88}x_8(t) + r_8$$

Lipids (fats) are stored in adipose tissue as triglycerides. These triglycerides are broken down (lipolysis) into glycerol and free fatty acids which are then free to be transported via the blood to other tissues and organs for utilization and conversion. Lipolysis

is thus the main source of FFA in systemic blood. It is well-known that a rise in plasma insulin results in a fall in plasma FFA [22], [23], this fall being attributed to a decrease in lipolysis [25], [34, p. 702] caused by an inhibitory effect on a triglyceride lipase [19], [25] involved in the breakdown of TG to FFA and glycerol. This effect of insulin on the lipase activity of adipose tissue is, in turn, thought to be a consequence of the reduction of tissue cAMP concentrations brought about by increased insulin levels [6], [24], [35] (see the above discussions on insulin and cAMP). The exact mechanisms by which cAMP controls lipase activity is not yet known. While the role of glucagon in enhancing lipolysis is somewhat less established, it appears that the lipolytic effect of glucagon is also mediated throught its effect on cAMP levels [6], [19]. Thus we have represented the diametrically opposed actions of insulin and glucagon by the first two terms in the equation above.

Studies of the effect of intraveneous insulin administration on blood glucose and FFA levels have shown that plasma FFA fall is a function almost exclusively of insulin levels [9, p. 94]. Thus in the model we have assumed that any effects that changing glucose concentrations might have on FFA levels are mediated entirely through insulin.

An increase in growth hormone is believed to produce two effects on plasma FFA levels: (i) an increased release of FFA into the blood due to enhanced lipolysis [25, p. 242], (ii) a lowering of plasma FFA due to increased muscle uptake (possibly because of increased fat oxidation in muscle) [32], [40]. Although both effects are believed to be important, the latter is thought to be an immediate effect, the former a delayed effect [25], [32], [33]. Rabinowitz, et. al. [32]

found that injections of growth hormone in human forearm tissues caused a prompt increase of FFA in muscle, an effect which they attributed to increased muscle oxidation because of an observed drop in respiratory quotient. Although their explanation has been debated because it has not been possible to demonstrate accelerated fatty acid oxidation in growth hormone treated animals [19: 3rd ed., p. 333], it does seem reasonable to accept their observations of increased uptake. Their work and that of others [17], [25], [33] reveal a delay of at least 40 minutes before the increase in lipolysis is observed. This delay is thought to be related to growth hormone stimulated synthesis of RNA and protein molecules [17]. Since, for most of our subjects, an increase of growth was observed only in the last hour of the OGTT, we have neglected this delayed effect of increased lipolysis in our model. It could be added to the term above representing increased uptake with little effort.

In addition to insulin, growth hormone, and glucagon, FFA plasma levels also appear to be affected by epinephrine, thyroxin, and glucocorticoids. The stimulating effect of epinephrine on lipase activity [23], [25] is naturally thought to be a consequence of increased cAMP tissue concentrations [6]. The inability to include the effects of epinephrine caused substantial difficulty in attempts to fit our model to FFA data curves in the period around 4 hours after ingestion (see Figure 3.5). One might expect to be unable to attain (with the model) the observed levels of FFA if one considers the likely possibility of increased release of epinephrine by the adrenal medulla during the recovery phase of the OGTT response.

Thyroxin and glucocorticoids are also thought to increase plasma levels of FFA [23], [27, p. 337], by enhancing lipolysis through their

effects on levels of a hormone-sensitive lipase [6], [19, p. 223], [25], [34, p. 704]. Since plasma levels of these hormones remain relatively constant during the OGTT, their effects are included in the term r_8.

Finally, we assume in the model that plasma FFA concentration is a stimulus for FFA uptake by liver and muscle for storage and FFA uptake by the liver for production and release of triglycerides and ketones.

Numerous simulations varying the parameters in the model have been carried out on an IBM 360 at Brown University. The results of one of these simulations is depicted in Figures 3.1 - 3.5 below by the unbroken lines. A set of data chosen from a representative normal subject is shown by the cross-marks on these graphs. (Although on this specific volunteer the quantitative glucagon data points were not all available, the data curve is, we feel, qualitatively correct in accordance with our experience with other data sets.) The paramter values used in the simulations graphed below are as follows:

Glucose:

$a_{127} = 10$ $\quad$ $a_{12} = .1$

$\alpha = 40$ $\quad$ $a_{13} = 70$

$A = 60$ $\quad$ $\alpha_{13} = 30$

$m = .6$ $\quad$ $\beta_{13} = 90$

$\alpha_{11} = 40$ $\quad$ $a_{18} = 30$

$\beta_{11} = 110$ $\quad$ $\alpha_{18} = .4$

$\alpha_{17} = .5$ $\quad$ $\beta_{18} = .5$

$\beta_{17} = 3$ $\quad$ $r_1 = -30$

Insulin:

$a_{21} = 13$ $\quad$ $\bar{r}_{21} = 35$

$r_{21} = 78$ $\quad$ $a_{22} = 6$

$\bar{a}_{21} = 1.5$ $\quad$ $r_2 = 70$

Glucagon:

$a_{31} = 200$ $\quad$ $a_{33} = 4$

$\alpha_{31} = 76$ $\quad$ $r_3 = 55$

$\beta_{31} = 110$

Growth hormone:

$a_{71} = 6$ $\quad$ $\bar{r}_{71} = 80$

$r_{71} = 85$ $\quad$ $a_{77} = 4.5$

$\bar{a}_{71} = .65$ $\quad$ $e_7 = 1.3$

Free fatty acid:

$a_{82} = .65$ $\quad$ $a_{87} = .76$

$\alpha_{82} = 40$ $\quad$ $\alpha_{87} = 2$

$\beta_{82} = 80$ $\quad$ $\beta_{87} = 18$

$a_{83} = .3$ $\quad$ $a_{88} = 1.5$

$\alpha_{83} = 50$ $\quad$ $r_8 = .6$

$\beta_{83} = 65$

This preliminary fit leaves room for improvement and it is clear that much work remains to be done. As more data is collected and the model is modified and improved, our confidence in it will undoubtedly grow.

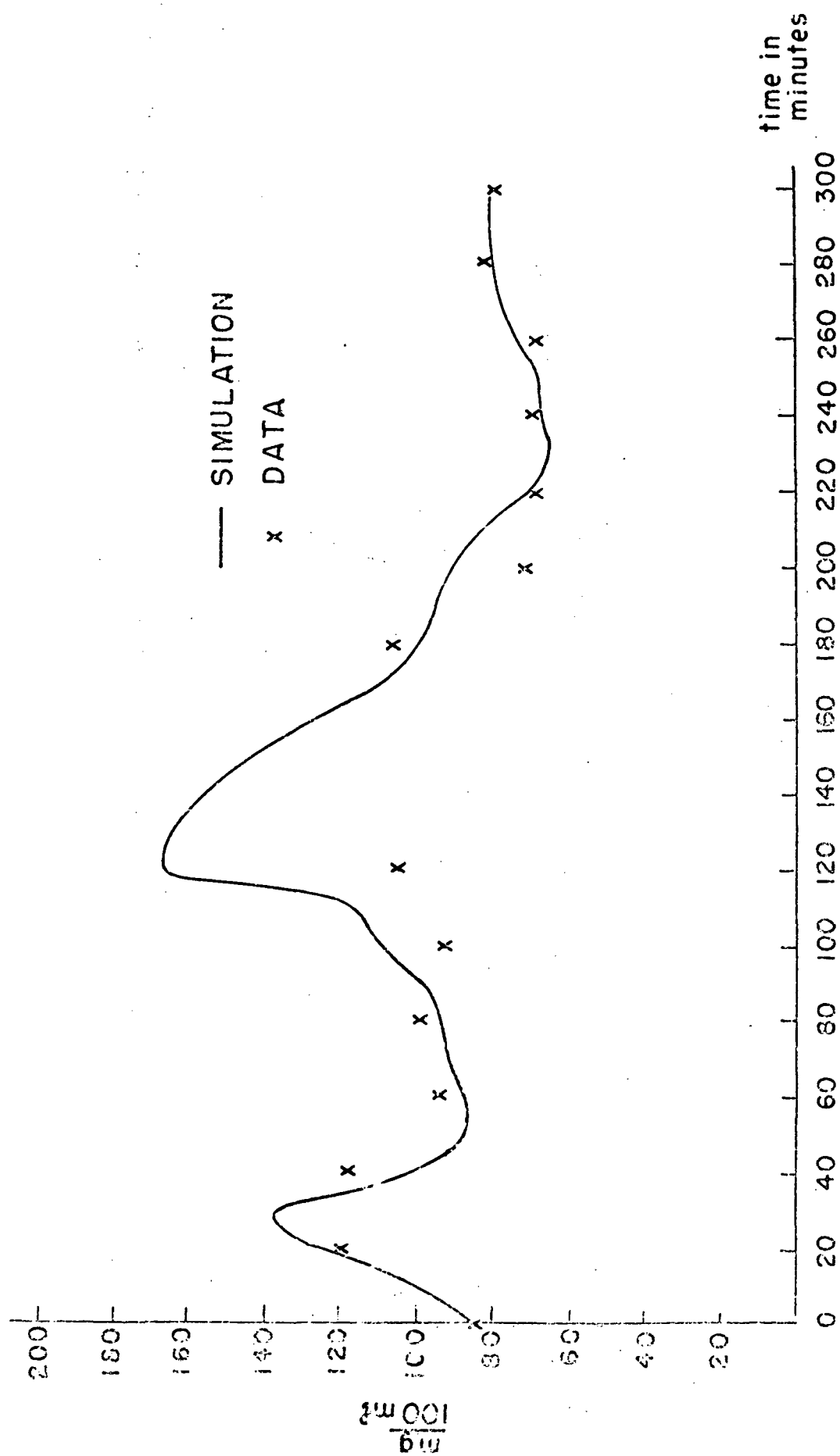
SIMULATION
DATA
GLUCOSE
time in minutes
mg/100 ml
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
20
40
60
80
100
120
140
160
180
200

Figure 3.1

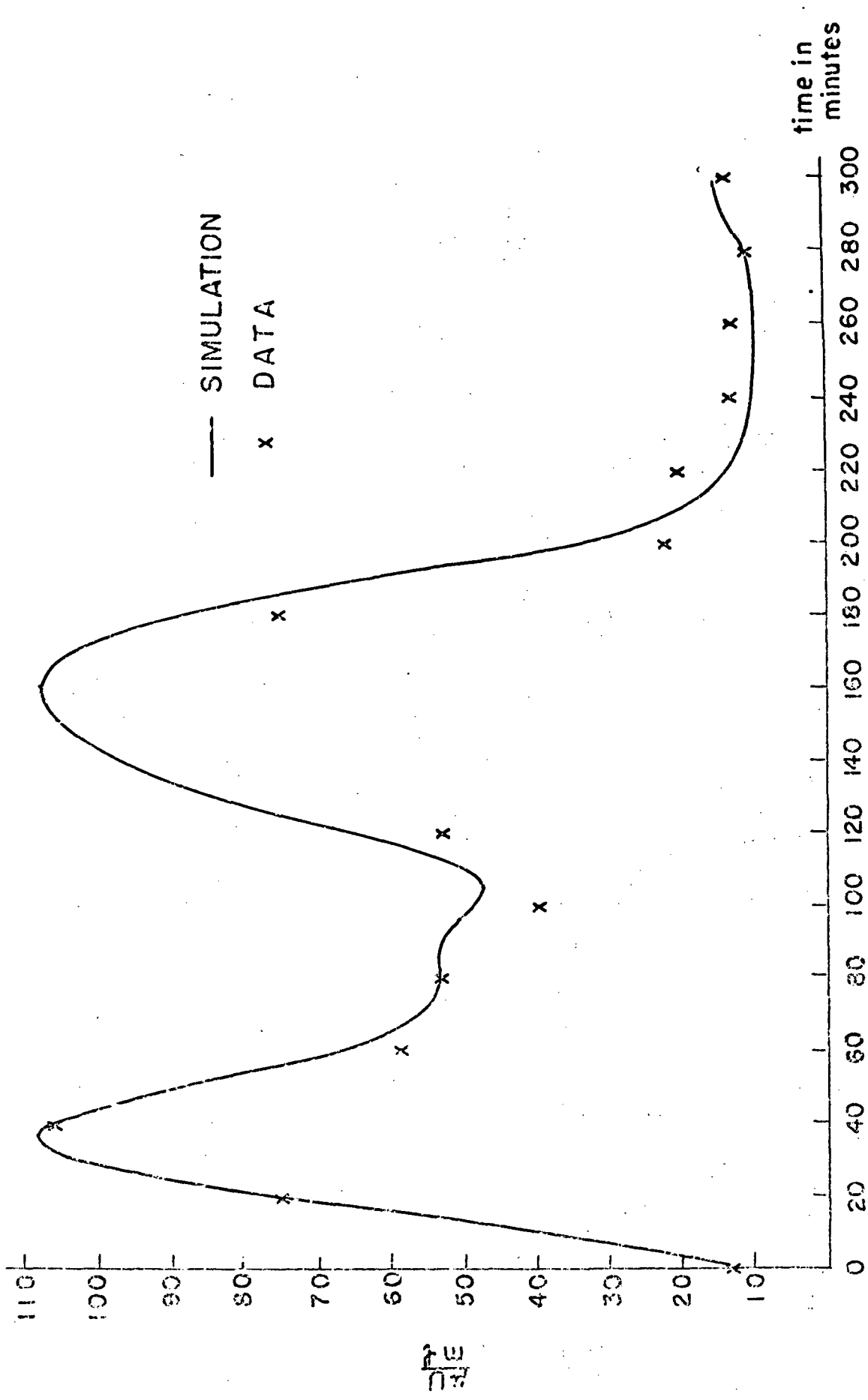
SIMULATION
DATA
time in minutes
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300
10 20 30 40 50 60 70 80 90 100 110
INSULIN

Figure 3.2

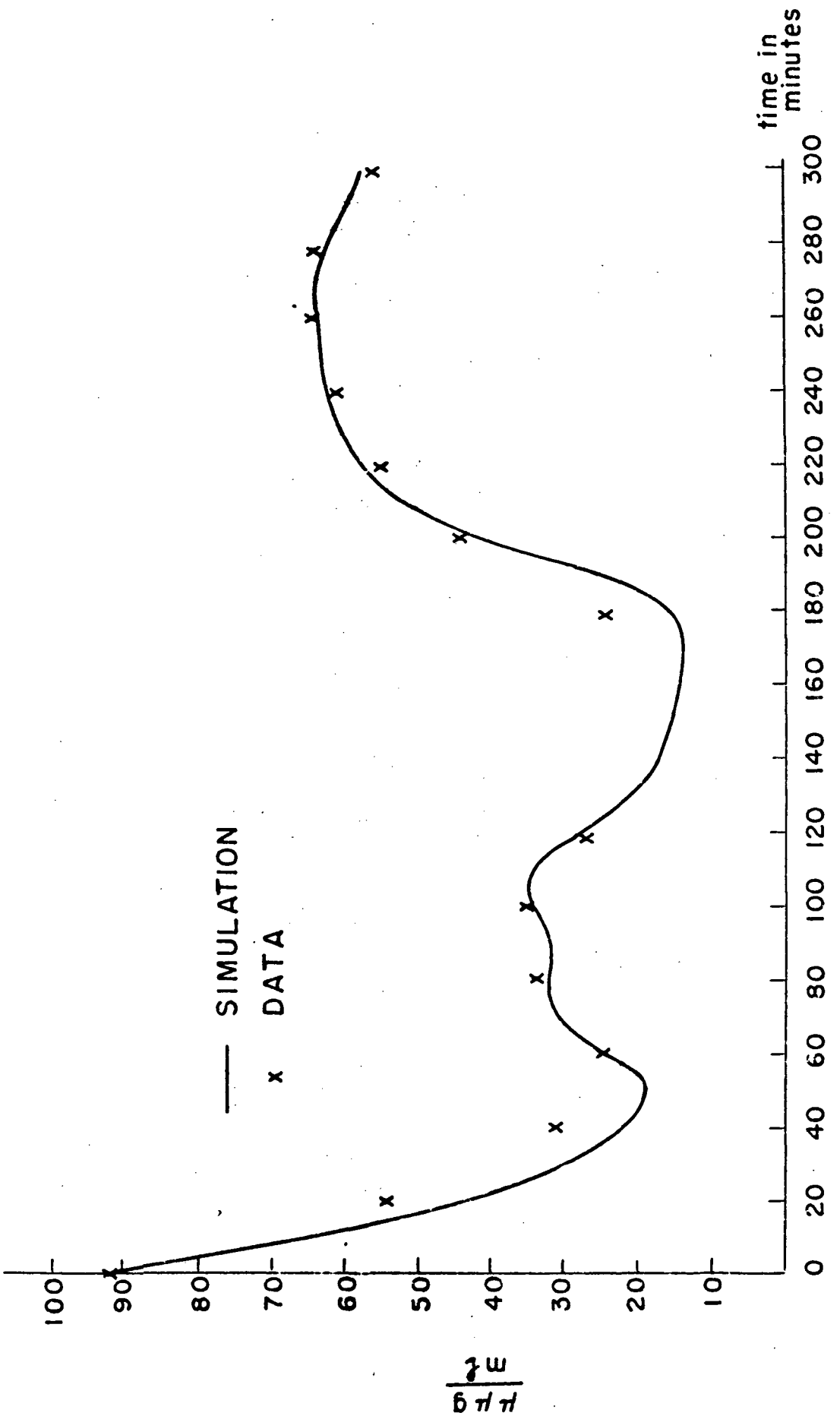
μμg/ml
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
time in minutes
SIMULATION
DATA
GLUCAGON

Figure 3.3

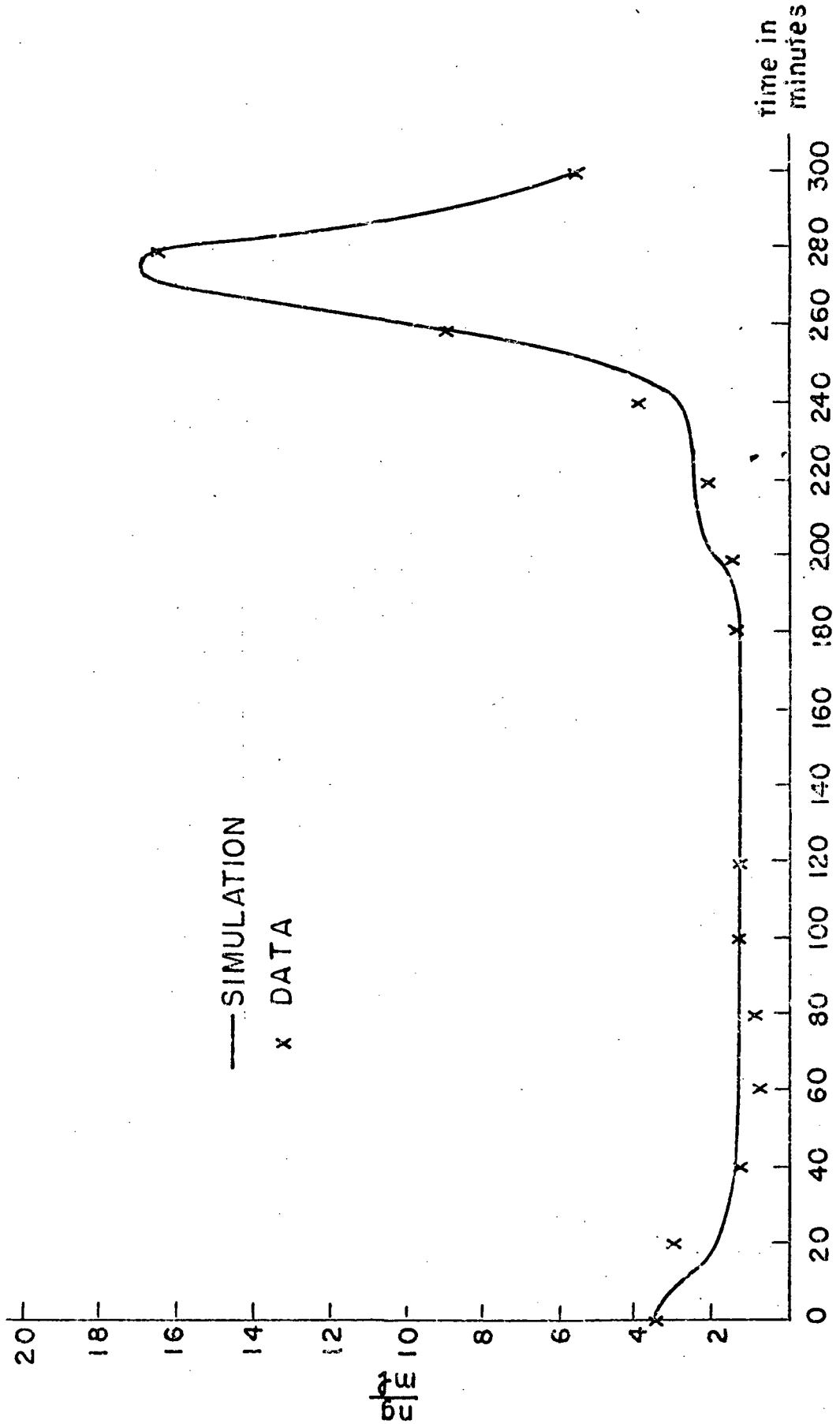
GROWTH HORMONE
—SIMULATION
x DATA
time in minutes
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
2
4
6
8
10
12
14
16
18
20

Figure 3.4

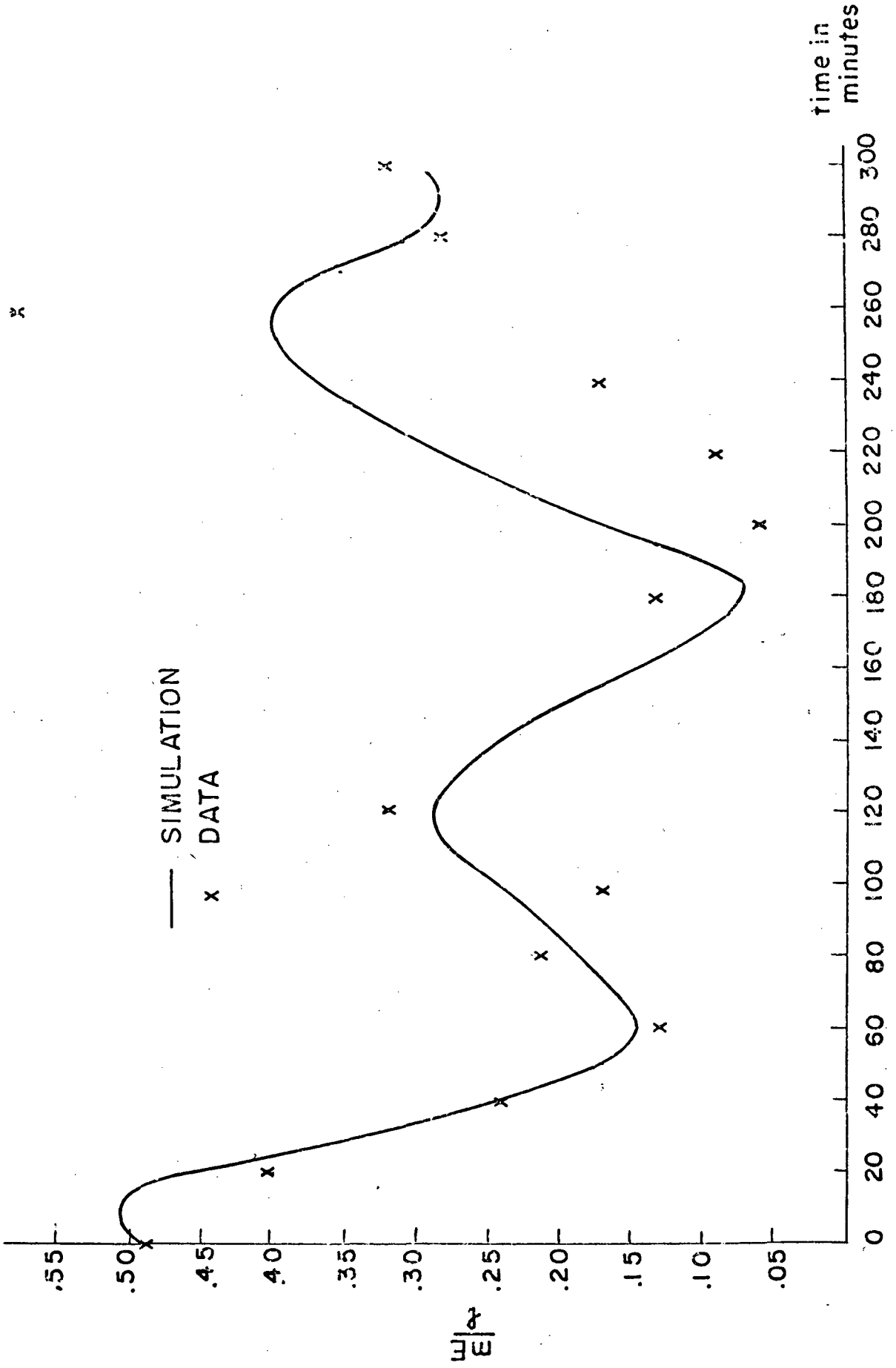
SIMULATION
DATA
FREE FATTY ACID
time in minutes
E/E_m
.55
.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300

Figure 3.5

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